Table 6. The biochemical identification of bacteria isolated from Galveston Bay, TX shellstock oysters irradiated at 0.0, 1.0 and 3.0 kGy using the API 20e™ Identification System of Enterobacteriaceae.

Treatment Group	Organism Identified	Identification Status	Media Isolated From
Control	Aeromonas hydrophila	Very Good ID	PCAS
	Citrobacter freundii	Excellent ID	PCAS
	Escherichia coli	Excellent ID	EC-MUG
	Proteus vulgaris	Excellent ID	PCA
	Salmonella spp.	Excellent ID	PCA
	Vibrio alginolyticus	Excellent ID	TCBS
	Vibrio fluvialis	Excellent ID	PCAS
	Vibrio parahaemolyticus	Excellent ID	TCBS
	Vibrio vulnificus	Excellent ID	TCBS/CPC
1.0 kGy	Aeromonas hydrophila	Very Good ID	PCAS
	Citrobacter freundii	Excellent ID	PCAS
	Escherichia coli	Excellent ID	EC-MUG
	Proteus vulgaris	Excellent ID	PCA
	Salmonella spp.	Excellent ID	PCA
	Vibrio alginolyticus	Excellent ID	TCBS
	Vibrio fluvialis	Excellent ID	PCAS
	Vibrio parahaemolyticus	Excellent ID	TCBS
	Vibrio vulnificus	Excellent ID	TCBS/CPC
3.0 kGy	Aeromonas hydrophila	Very Good ID	PCAS
	Citrobacter freundii	Excellent ID	PCAS
	Enterobacter gergoviae	Excellent ID	PCA
	Escherichia coli	Excellent ID	EC-MUG
	Pseudomonas pseudomallei	Very Good ID	PCA
	Vibrio alginolyticus	Excellent ID	TCBS
	Vibrio parahaemolyticus	Excellent ID	TCBS
	Vibrio vulnificus	Excellent ID	TCBS/CPC

The other organisms identified consisted again of Aeromonas spp. and Citrobacter spp. with an Enterobacter gergoviae and Pseudomonas pseudomallei organism identified at only the 3.0 kGy dose. Perhaps some other of bacteria were inhibiting these two before irradiation at 3.0 kGy, and thus these two resistant organisms were then able to grow and divide without competition from others after irradiation.

Internal Dosimetry of Shellstock Oysters

Data from this experimentation was obtained only on a 4 dosimeter strips, but the data was clear. The dosimeters used inside of the oysters exposed to 1.0 kGy recorded a dose of only 0.5 kGy, while the dosimeters in the 3.0 kGy oysters only recorded 1.6 kGy. This is critical because the internal meat structure is not receiving the same dose that the dosimeters outside of the box are recording. Thus, bacterial destruction will not as great.

Determination of V. vulnificus D_{10} Value in ASW

This phase of the experimentation consisted of determining the D_{10} values for logarithmic phase, stationary phase, and VBNC forms of V. vulnificus. These values are critical in determining the radiation dose necessary for control of this bacteria, depending on growth phase or nonculturability and growth media.

Stationary phase (O, T and B) D_{10} Value in ASW

The D_{10} values of stationary phase V. vulnificus cells are depicted in Figures 18-20. These figures show the linear regression analysis (best fit straight line) of the irradiation of stationary phase V. vulnificus. The R^2 and equation of the line are shown for the CPC agar only, as well as the D_{10} calculation. CPC agar was selected because of its high selectivity for V.

vulnificus compared to the other media. In Figure 18, the D_{10} value associated with the O morphotype in ASW was determined to be D_{10} = 0.055 kGy on TCBS agar, D_{10} = 0.059 kGy on CPC agar and D_{10} = 0.057 kGy on APA agars. These values coincide quite closely with Dixon (1992) who found the D_{10} = 0.062 kGy for an 18-24 hour O V. vulnificus in phosphate buffered saline. The R^2 was found to be 0.981 and the equation of the line was calculated as y = -17.04x + 122.5 on the CPC agar.

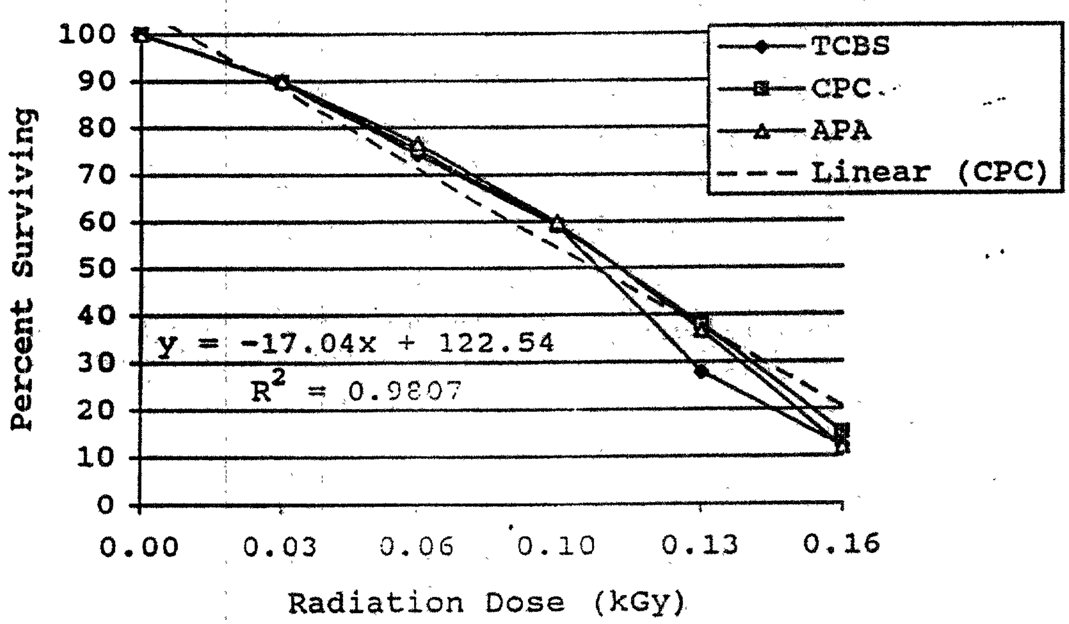

Figure 18. Plot of surviving fraction versus radiation dose for stationary phase "O" Vibrio vulnificus C7184 in ASW, plated on TCBS (D_{10} = 0.055 kGy), CPC (D_{10} = 0.059 kGy) and APA (D_{10} = 0.057 kGy) agars.

In Figure 19, the D_{10} value associated with the "blue-bug" or mutant, which is an O morphotype, in ASW was determined to be D_{10} = 0.056 kGy on TCBS agar, D_{10} = 0.057 kGy on CPC agar and D_{10} = 0.057 kGy on APA agar and D_{10} = 0.057 kGy on TN agar. These values again coincide with Dixon (1992) who found the D_{10} = 0.062 kGy for an 18-24 hour O V. vulnificus in phosphate buffered saline. The R^2 was found to be 0.977 and the equation of the line was calculated as y = -17.52x + 123.3 on the CPC agar.

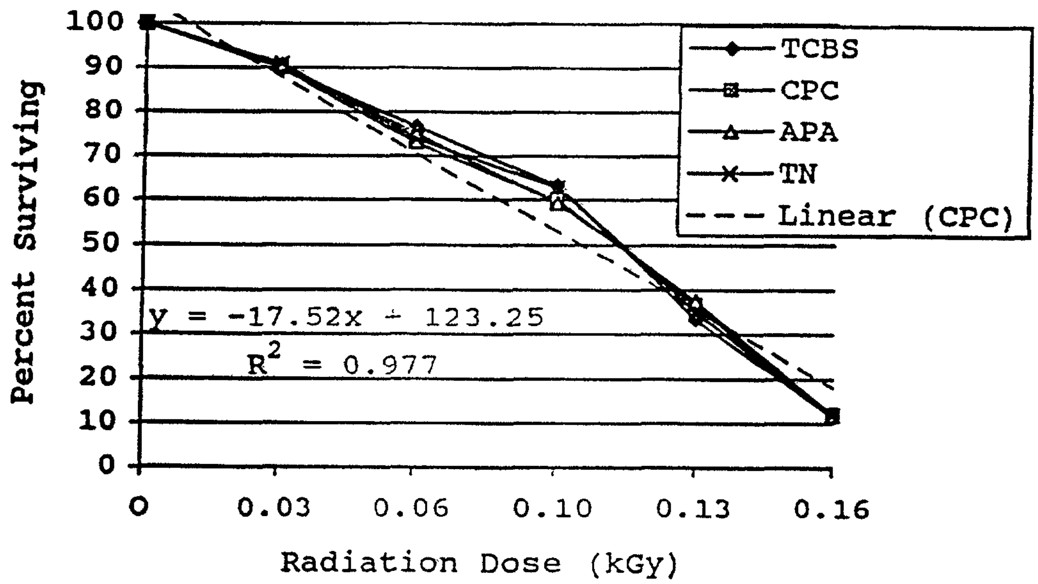

Figure 19. Plot of surviving fraction versus radiation dose for stationary phase "B" Vibrio vulnificus CVD 713 in ASW, plated on TCBS (D_{10} = 0.056 kGy), CPC (D_{10} = 0.057 kGy), APA (D_{10} = 0.057 kGy) and TN (D_{10} = 0.057 kGy) agars.

In Figure 20, the D_{10} value associated with the T morphotype in ASW was determined to be D_{10} = 0.045 kGy on TCBS agar, D_{10} = 0.043 kGy on CPC agar and D_{10} = 0.044 kGy on APA agars. These values are just slightly higher than those reported by Dixon (1992) who found the D_{10} = 0.037 kGy for an 18-24 hour T V. vulnificus in phosphate buffered saline. The R^2 was found to be 0.913 and the equation of the line was calculated as y = -23.26x + 131.1 on the CPC agar.

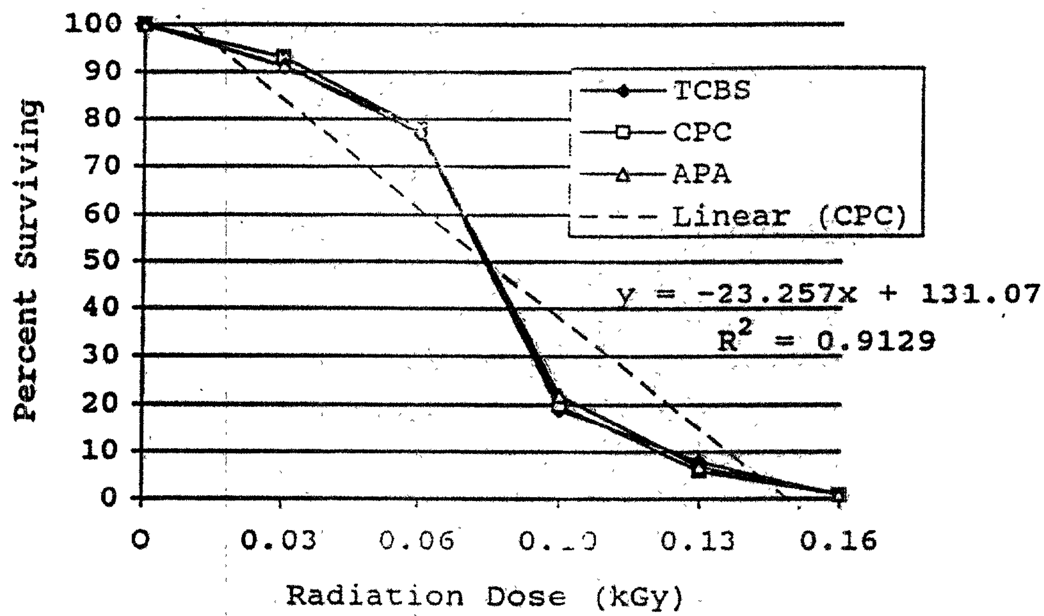

Figure 20. Plot of surviving fraction versus radiation dose for stationary phase "T" Vibrio vulnificus C7184 in ASW, plated on TCBS (D_{10} = 0.045 kGy), CPC (D_{10} = 0.043 kGy) and APA (D_{10} = 0.044 kGy) agars.

Logarithmic phase (O, T and B) D_{10} Value in ASW

The D_{10} values of logarithmic phase V. vulnificus cells are depicted in Figures 21-23. These figures show the linear regression analysis (best fit straight line) of the irradiation of all forms of V. vulnificus. The R^2 and equation of the line are shown for the CPC agar only, as well as the D_{10} calculation. CPC agar was selected because of its high selectivity for V. vulnificus compared to the other media. In Figure 21, the D_{10} value associated with the O morphotype in ASW was determined to be D_{10} = 0.054 kGy on TCBS agar, D_{10} = 0.053 kGy on CPC agar and D_{10} = 0.053 kGy on APA agars. These values coincide quite closely with Dixon (1992) who found the D_{10} = 0.062 kGy for an 18-24 hour O V. vulnificus in phosphate buffered saline. The R^2 was found to be 0.993 and the equation of the line was calculated as y = -18.86x + 123.2 on the CPC agar.

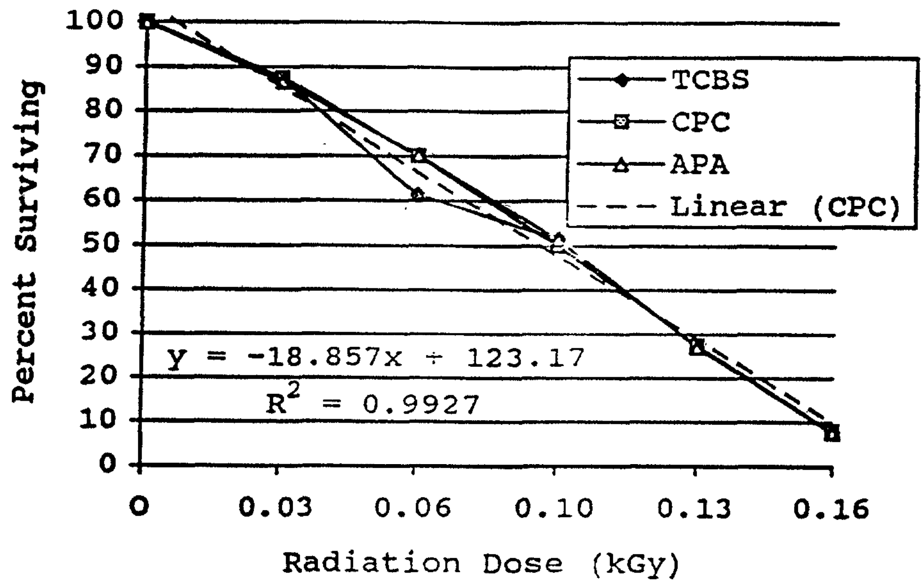

Figure 21. Plot of surviving fraction versus radiation dose for log phase "O" *Vibrio vulnificus* C7184 in ASW, plated on TCBS (D_{10} = 0.054 kGy), CPC (D_{10} = 0.053 kGy) and APA (D_{10} = 0.053 kGy) agars.

In Figure 22, the D_{10} value associated with the "blue-bug" in ASW was determined to be D_{10} = 0.053 kGy on TCBS agar, D_{10} = 0.054 kGy on CPC agar, D_{10} = 0.053 kGy on APA agar and D_{10} = 0.054 kGy on TN agar. These values again coincide with Dixon (1992) who found the D_{10} = 0.062 kGy for 18-24 hour O *V. vulnificus* in phosphate buffered saline. The R^2 was found to be 0.995 and the equation of the line was calculated as y = -18.69x + 121.9 on the CPC agar.

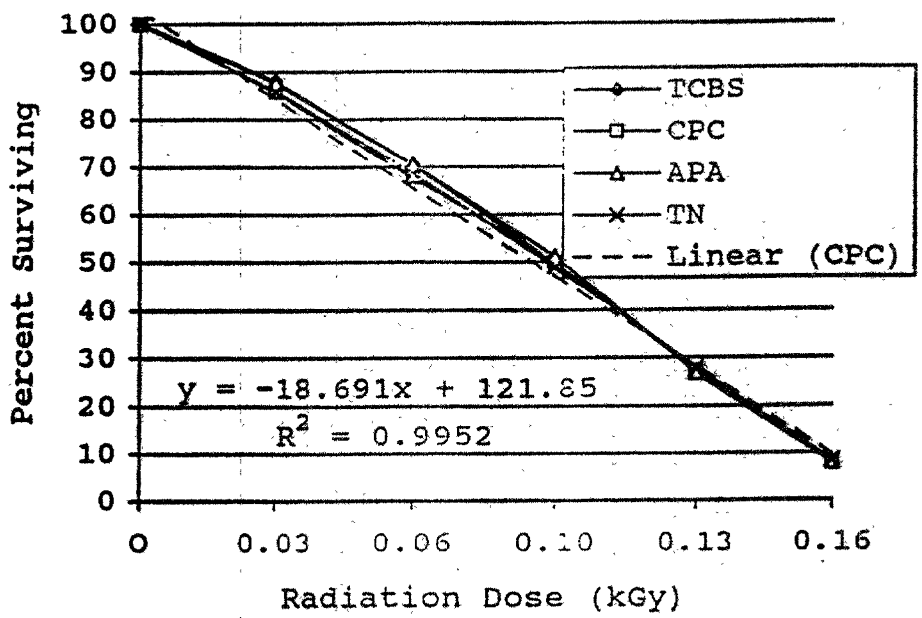

Figure 22. Plot of surviving fraction versus radiation dose for log phase "B" Vibrio vulnificus CVD 713 in ASW, plated on TCBS (D_{10} = 0.053 kGy), CPC (D_{10} = 0.054 kGy), APA (D_{10} = 0.053 kGy) and TN (D_{10} = 0.054 kGy) agars.

In Figure 23, the D_{10} value associated with the T morphotype in ASW was determined to be D_{10} = 0.043 kGy on TCBS agar, D_{10} = 0.043 kGy on CPC agar and D_{10} = 0.043 kGy on APA agars. These values are just slightly higher than those reported by Dixon (1992) who found the D_{10} = 0.037 kGy for an 18-24 hour T V. vulnificus in phosphate buffered saline. The R^2 was found to be 0.909 and the equation of the line was calculated as y = -23.17x + 129.3 on the CPC agar.

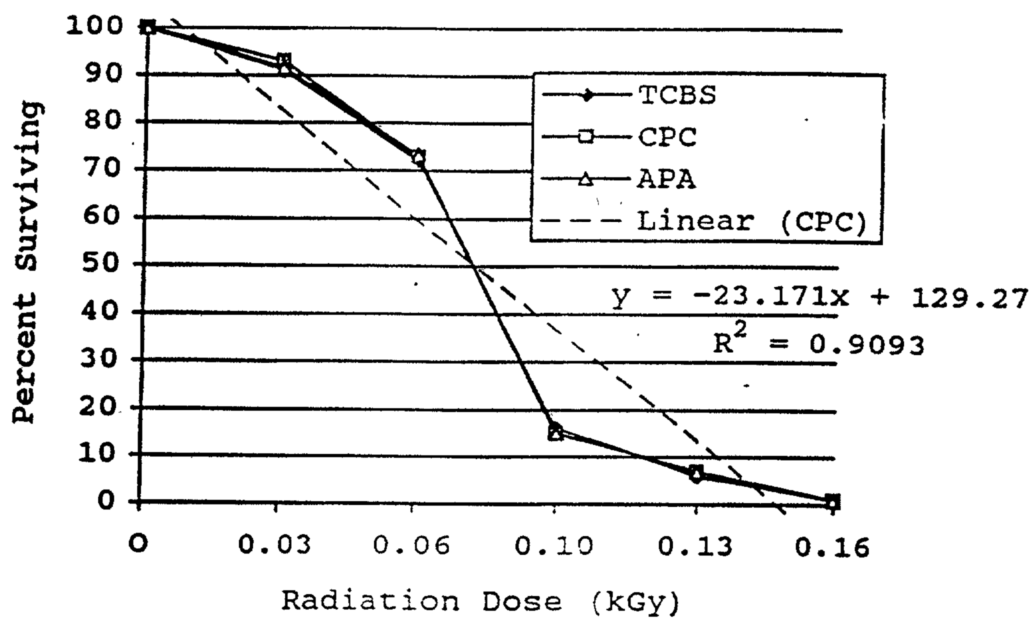

Figure 23. Plot of surviving fraction versus radiation dose for log phase "T" Vibrio vulnificus C7184 in ASW, plated on TCBS (D_{10} = 0.043 kGy), CPC (D_{10} = 0.043 kGy) and APA (D_{10} = 0.043 kGy) agars.

VBNC (O, T and B) D_{10} Value in ASW

The D_{10} values of VBNC V. vulnificus cells (O, T and B) are depicted in Figures 24-26. These figures show the linear regression analysis (best fit straight line) of the irradiation of VBNC V. vulnificus. The R^2 and equation of the line are shown for the DVC only, as well as the D_{10} calculation. This is presented first, because immediately after irradiation, the only way to count VBNC cells is by the direct viable count, otherwise a 24-48 hour resuscitation is required before growth can be detected on microbiological media. In Figure 24, the D_{10} value of the VBNC O morphotype cells was determined to be 0.165 kGy. The equation of the line generated from the death curve is $y = -6.06x + 107.76$, with a corresponding

R^2 value of 0.979. This D_{10} value is 3 times larger than what was observed with the normal cells irradiated in ASW, indicating that the VBNC state provides increased radioresistance for the organism.

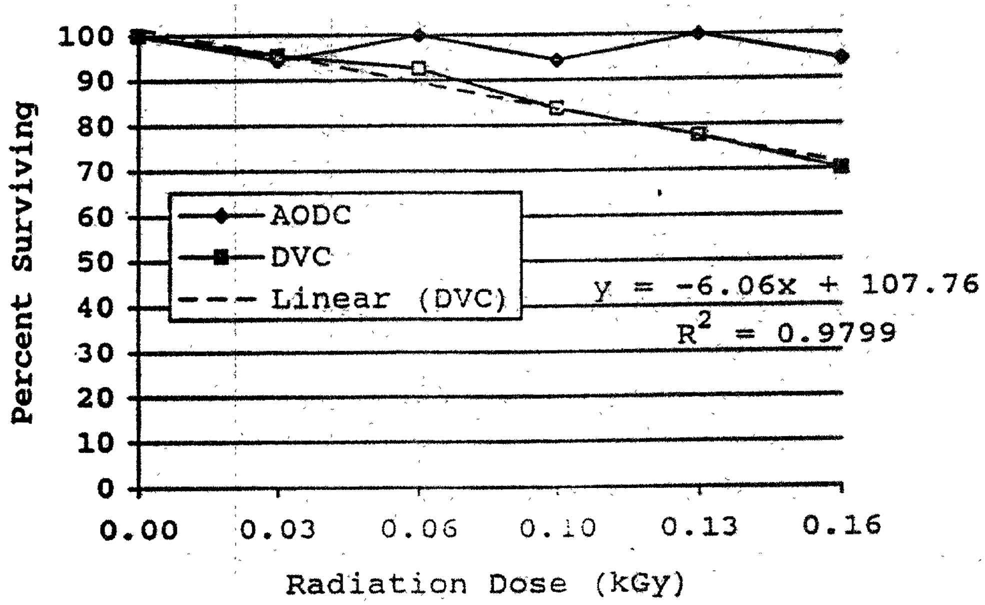

Figure 24. Plot of surviving fraction versus radiation dose for VBNC "O" Vibrio vulnificus C7184 in ASW, as detected by DVC (D_{10} = 0.165 kGy) and AODC.

In Figure 25, the D_{10} value of the VBNC "blue-bug" (B), which is an O morphotype cell, was determined to be 0.173 kGy, which is very close to the 0.165 kGy observed with the O morphotype in Figure 24. The equation of the line generated from the death curve is y = -5.75x + 108.47, with a corresponding R^2 value of 0.957. This D_{10} value is more than 3 times larger than what was observed with the normal cells irradiated in ASW, indicating again that the VBNC state provides increased radioresistance for the organism.

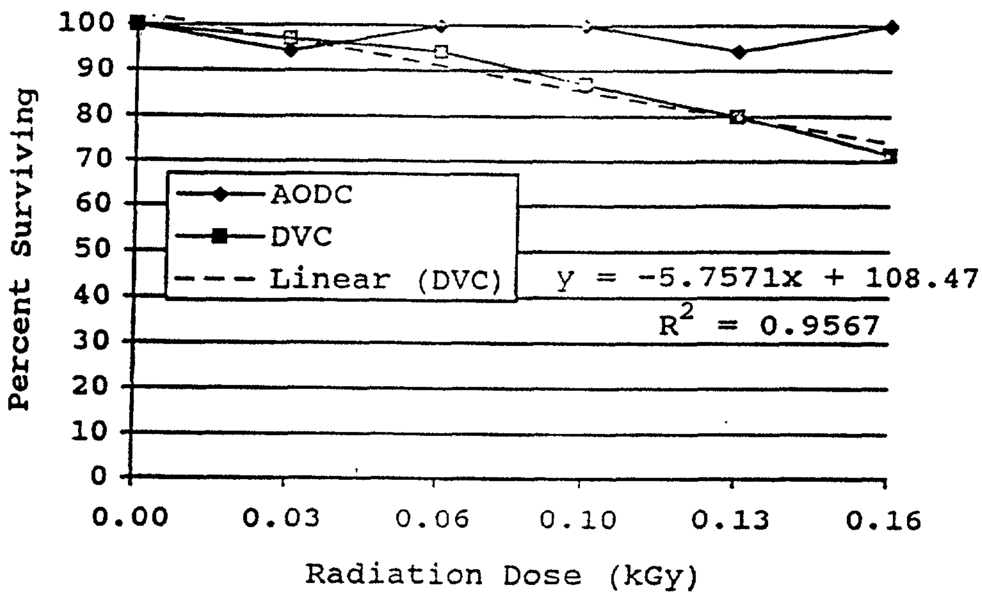

Figure 25. Plot of surviving fraction versus radiation dose for VBNC "B" Vibrio vulnificus CVD 713 in ASW, as detected by DVC (D_{10} = 0.173 kGy) and AODC.

The trend of increased radioresistance in the VBNC cells is continued with the T morphotype. In Figure 26, the D_{10} value of the VBNC T morphotype cells was determined to be 0.147 kGy. The equation of the line generated from the death curve is $y = -6.83x + 107.78$, with a corresponding R^2 value of 0.988. This D_{10} value is 3 times larger than what was observed with the normal cells irradiated in ASW, indicating that even with the nonencapsulated cells, the mechanisms that lead to nonculturability, also help provides increased radioresistance for the organism.

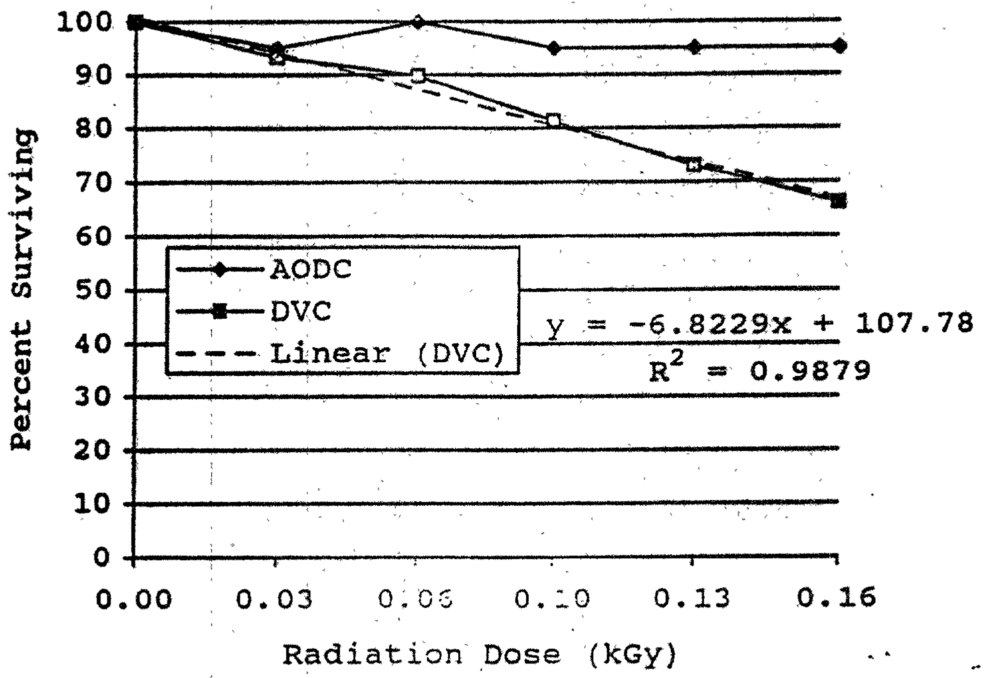

Figure 26. Plot of surviving fraction versus radiation dose for VBNC "T" Vibrio vulnificus C7184 in ASW, as detected by DVC (D_{10} = 0.147 kGy) and AODC.

Resuscitation of VBNC Post-Irradiation

Samples of viable but nonculturable cells of V. vulnificus that were irradiated at different doses were also incubated at room temperature for 24 and 48 hours and checked for resuscitation post-irradiation. Figures 27-32 depict the resuscitation profiles of irradiated O, T and B VBNC V. vulnificus cells. In Figure 27, 24 hours after irradiation, culturability begins to return close to the DVC values of nearly 1 x 10^5 in the HIS and APA plates for the O C7184 strain, however the more selective TCBS and CPC plates are about 0.5 logs lower. This is seen at all doses, including zero dose.

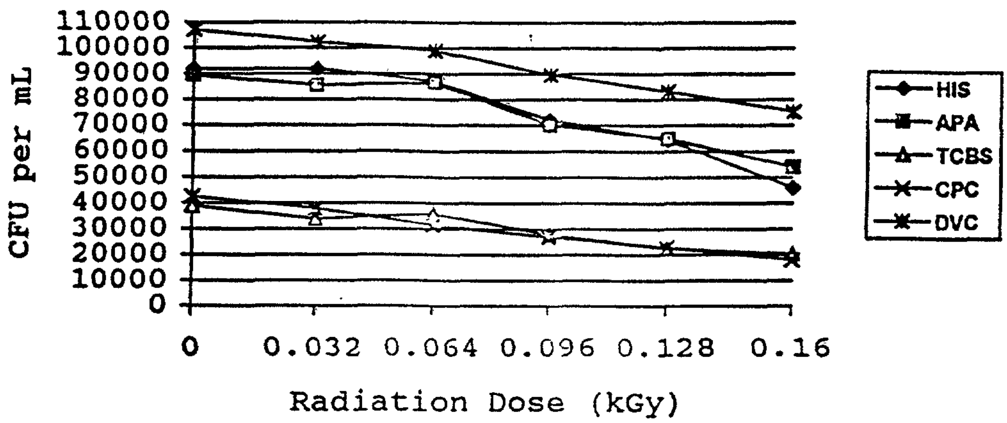

Figure 27. Room temperature (25°C) resuscitation of irradiated VBNC "O" <u>Vibrio vulnificus</u> in ASW after 24 hours and plating on HIS, TCBS, CPC, APA and a DVC.

In Figure 28, it is shown that culturability begins to return close to the DVC values of nearly 1 x 10^5 in the HIS and APA plates for the mutant B CVD 713 after 24 hours of room temperature incubation. However, the more selective TN, TCBS and CPC plates are still about 0.5 logs lower. This is again seen at all doses including zero dose.

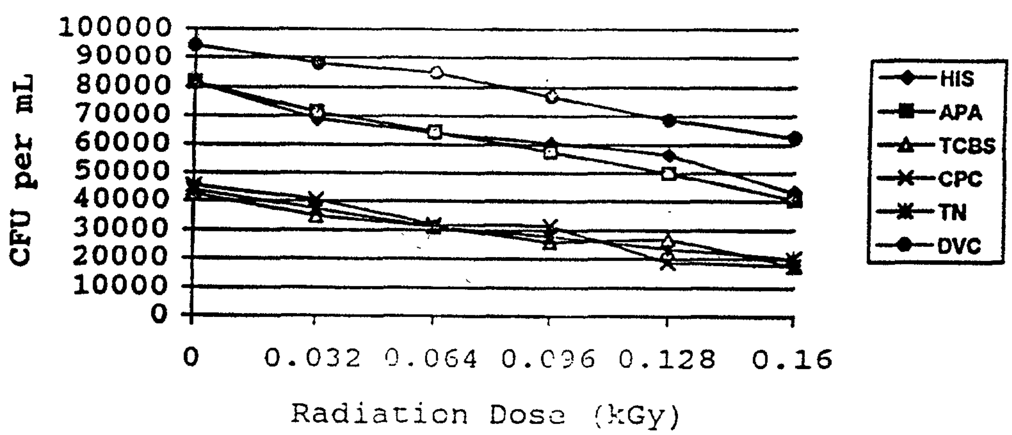

Figure 28. Room temperature (25°C) resuscitation of irradiated VBNC "B" <u>Vibrio vulnificus</u> in ASW after 24 hours and plating on HIS, TCBS, CPC, TN, APA and a DVC.

In Figure 29, 24 hours after irradiation, culturability begins to return close to the DVC values of nearly 1 x 10^5 in the HIS and APA plates for the T C7184 strain, however the more selective TCBS and CPC plates are also about 0.5 logs lower. This trend is observed at all doses.

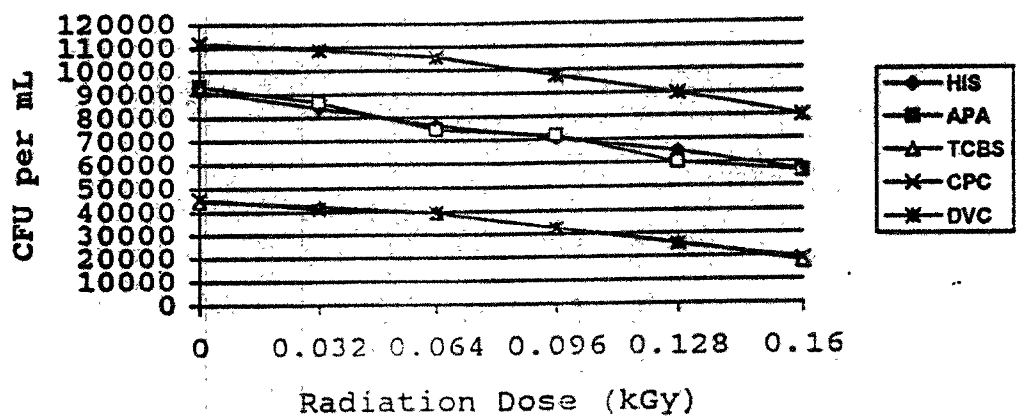

Figure 29. Room temperature (25°C) resuscitation of irradiated VBNC "T" *Vibrio vulnificus* in ASW after 24 hours and plating on HIS, TCBS, CPC, APA and a DVC.

Data in Figure 30 show that culturability returns to the direct viable count values for the O C7184 strain of over 1 x 10^5 in the HIS and APA plates, as well as the more selective TCBS and CPC plates, 48 hours after irradiation. The numbers obtained on all media are nearly equal to the DVC indicating that complete resuscitation has occurred. It should be noted that the plate counts never exceeded the DVC showing that resuscitation of VBNC cells occurred and not the growth of a few culturable cells.

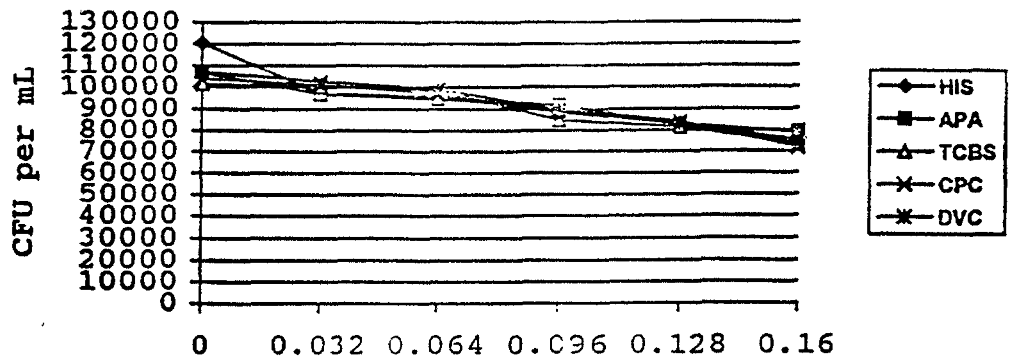

Figure 30. Room temperature (25°C) **resuscitation of irradiated** VBNC "O" <u>Vibrio vulnificus</u> **in ASW after 48 hours and plating** on HIS, TCBS, CPC, **APA and a DVC.**

In Figure 31, the data show that **culturability also returns to the** levels of the direct viable count **values for the B CVD** 713 strain of over 1 x 10^5 **on the HIS, APA, TN, TCBS and** CPC plates, 48 hours after **irradiation,.**

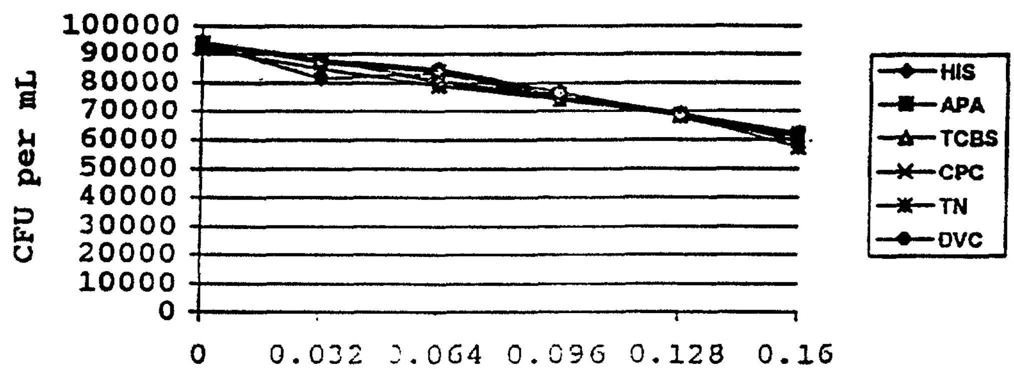

Figure 31. Room temperature (25°C) **resuscitation of irradiated** VBNC "B" <u>Vibrio vulnificus</u> **in ASW after 48 hours and plating** on HIS, TCBS, CPC, **TN, APA and a DVC.**

The numbers obtained on all media are **again nearly equal to the** DVC indicating that complete **resuscitation has occurred.** Similarly, the plate counts **never exceeded the**

DVC showing that resuscitation of VBNC cells occurred, and not the growth of a few culturable cells.

In Figure 32, 48 hours after irradiation, culturability returns all the way up to the direct viable count values for the T C7184 strain of over 1 x 10^5 on HIS, APA, TCBS and CPC plates. The numbers again indicate resuscitation as they are nearly equal to the DVC, without ever exceeding the DVC showing that resuscitation of VBNC cells occurred, and not the growth of a few culturable cells.

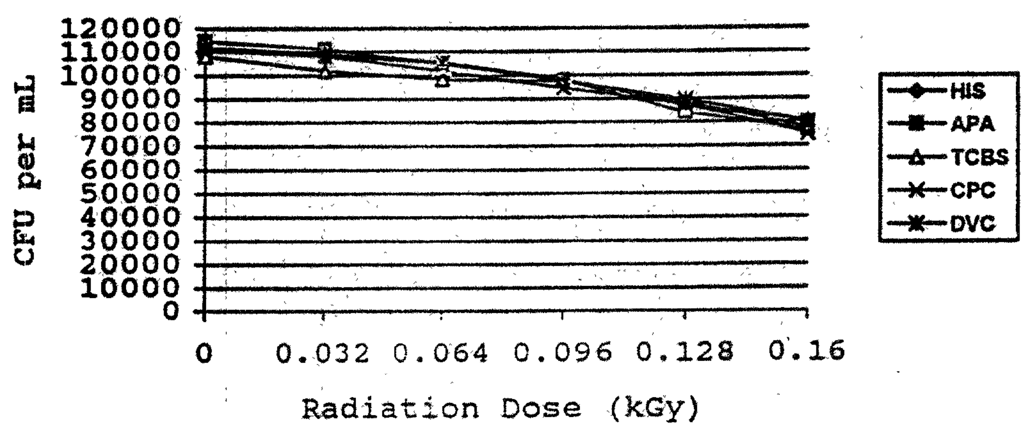

Figure 32. Room temperature (25°C) resuscitation of irradiated VBNC "T" *Vibrio vulnificus* in ASW after 48 hours and plating on HIS, TCBS, CPC, APA and a DVC.

There is a lot of criticism involving the resuscitation of cells. There is another school of thought claiming that true resuscitation does not really occur (except for maybe a few cells). Rather the increase in bacterial numbers seen on plating media is due to the growth and division of either a few cells that have resuscitated, or a few cells that remained culturable all along.

To address this controversy, the easiest method to answer the question is by the addition of nalidixic acid to resuscitating cells. In this case, any culturable cells present in the sample would be inhibited by the nalidixic acid in terms of DNA replication and hence cell division. The nonculturable cells would then be the only organisms under consideration. If they truly were resuscitated, then once the 24-48 hours of room temperature resuscitation passed, they alone would be the source of the positive growth on HIS, APA, TN, TCBS or CPC agars.

A separate set of irradiation experiments were conducted to determine the effects of adding nalidixic acid to the resuscitation medium after irradiation. It was determined in the presence of nalidixic acid, irradiated VBNC cells (O, T and B) resuscitated at room temperature, for 48 hours, reached levels equal to the original value determined in the DVC. No graph is presented for this finding as the parameters for inducing the VBNC state and irradiation protocol were followed identically to the that listed previously, and thus all of the numbers were very similar to the numbers presented in Figures 27-32. The findings of this research indicate that true resuscitation occurred after the irradiation of

VBNC cells (O, T and B), and not the regrowth of culturable cells.

Other influences on resuscitation can be the microbial concentration in the sample, as well as the resuscitation medium. Two separate experiments were designed to address this issue. First, after the irradiation of VBNC cells (O, T and B), 2.0 mL undiluted samples were allowed to resuscitate at room temperature for 48 hours, as well as 10-, 100- and 1000-fold dilutions. The undiluted samples resuscitated back to the original DVC values, but none of the diluted samples resuscitated.

The effects of resuspension media were also assessed. A whole 750 mL microcosm was spun down, yielding a pellet. This pellet was washed 2X and subsequently resuspended in fresh ASW. The result was complete resuscitation as was seen before. However, when this fresh resuspension was diluted 10-, 100- and 1000-fold, no resucitation occurred. The first possibility is that the ASW has nothing to do with the resuscitation, as resuscitation occurred in both the fresh and old ASW. However, there is some concentration effect, since diluted samples of the VBNC microcosms did not resuscitate. Perhaps the organisms must be present in a specific concentration so that they can produce some factor which promotes resuscitation. Perhaps there are compounds already present in the microcosm, and they are captured along with the cells during centrifugation. If so, compounds could be transferred with the cells to new seawater. A dilution of these cells and compounds may result in levels that are not usable by the bacteria. It seems that the cells are not as dependent on the ASW environment as they are on each other.

SUMMARY AND CONCLUSIONS

One of the primary objectives of this research was to determine the effects of ionizing radiation on FL and TX oysters on a true commercial scale in terms of shelf life (D_{20} and D_{50} values) and the microbiological consequences. Furthermore, these shellstock oysters were evaluated in terms of shell to meat ratio, internal shell dosimetry and the identification of any organisms surviving irradiation for up to 14 days in dry cold storage. The other aspect of the research dealt with determining the D_{10} values of the logarithmic and stationary phase of V. vulnificus strains C7184 (O, T) and mutant CVD 713 (B) in ASW and on a variety of media The VBNC form of these three V. vulnificus forms was also induced and the subsequent D_{10} values calculated for the VBNC cells using the direct viable count. In addition, the effects of radiation on 24 and 48 hour room temperature resuscitation was monitored on a variety of solid media and compared to the corresponding DVC. Also, an assessment was made as to whether or not true resuscitation occurred or whether the growback of few remaining culturable cells occurred. Furthermore, the effects of the type of media used for resuscitation (old or fresh ASW) was evaluated, as was the effect of different microbial concentrations on resuscitation.

In terms of shelf life, this research shows that the D_{20} values for FL shellstock oysters were 17, 4 and 2 days for the 0.0, 1.0 and 3.0 kGy exposures, respectively. The D_{20} values for TX shellstock oysters were 14, 4 and 2 days for the 0.0, 1.0 and 3.0 kGy exposures, respectively. The D_{50} values for FL shellstock oysters were >25, 9 and 3 days for the 0.0, 1.0 and 3.0 kGy exposures, respectively, whereas the D_{50} values for TX shellstock oysters were >25, 11 and 4 days for the 0.0, 1.0 and 3.0 kGy exposures, respectively. It is apparent that there is a serious decrease in shelf life associated with the irradiated oysters and that a serious product loss can be expected with irradiating summer oysters.

However, if the microbial levels are reduced to undetectable or very low levels, then the benefit of clean oysters may outweigh the rapid product loss. However, this is not the case. Immediately following irradiation, there is a decrease of about 2 logs in total bacterial numbers and Vibrio levels regardless of source or dose. However, this lower value is only maintained for a few days and then the bacterial numbers begin to rise back up to the nonirradiated, control values or even higher. The oysters are dying rapidly and the bacteria (total plate count, Vibrios and fecals) are multiplying rapidly. There are then very few days allowed for transport, stoarge, sale and consumption of irradiated oysters before they become unfit or die. This research also revealed the surviving organisms post-irradiation to be genera such as Vibrio spp., E. coli, Aeromonas and a few spoilage organisms such as Pseudomonas and Serratia. Furthermore, it was determined that the shell to meat ratios of FL, TX and LA oysters were 4.8, 5.5 and 7.2, respectively. Perhaps the most interesting piece of data surrounded the determination of the internal doses recieved inside the shell itself. The D_{max}/D_{min} ratios of 2:1 and 1.5:1 for the 1.0 kGy and 3.0 kGy exposures as detected by FTS, did not correlate well with the data obtained from the internal dosimters. It is quite clear that the internal part of the oyster received about half of the dose that the outside of the box received and that the density (shell to meat ratio is an indication) of the shellstock oysters is leading to true attenuation and thus a decreased dose. This, probably helps to explain the variability experienced when testing the oysters microbiologically as certain oysters are not getting the same internal dose and thus not the same bactericidal killing power. These "less-irradiated" oysters probably lead to the elevated counts sometimes experienced during dry storage sampling.

The other phase of experimentation dealt with the organism, V. vulnificus. The D_{10} values of (O, T and B)

V. vulnificus were determined both in the logarithmic and stationary phase in ASW and plated on a variety of media. The D_{10} values are displayed in Table 7.

Table 7. D_{10} values for log and stationary phase V. vulnificus on APA, TCBS, CPC and TN agars.

	APA	TCBS	CPC	TN
O Stationary	0.057	0.055	0.059	--
O Logarithmic	0.053	0.054	0.053	--
B Stationary	0.057	0.056	0.057	0.057
B Logarithmic	0.053	0.053	0.054	0.054
T Stationary	0.044	0.045	0.043	--
T Logarithmic	0.043	0.043	0.043	--

These values correspond quite well with Dixon (1992) with the O morphotype having a bit lower D_{10} in ASW than PBS and the T morphotype a bit higher in ASW than in PBS. As expected the mutant B type responded similarly to the the O morphotype, since in fact it is a genetically altered O type. No significant difference could be detected between the different types of media used in the growth after irradiation. Furthermore, there was no significant difference detected between the stationary and log phases. The D_{10} values were calculated for the VBNC form of these cells and they are displayed in Table 8. These D_{10} values for the VBNC cells are a full three times larger than what is observed for the normal, culturable cells. This indicates that the induction of the VBNC mechanisms of survival help provide an increased radioresistance fo the organism. This is most likely accomplished in the bacteria by reductive division whereby there is a compacting of the nuclear region, DNA rearrangement and a lessening of DNA per cell (Brauns et al., 1991). If this is the case, the probability of a hit decreases with decreasing nucleus size. Furthermore, compacted DNA would be wound so tight that the backbone structure would have less area exposed for a hit of gamma rays.

Table 8. The D_{10} values determined for VBNC cells of *V. vulnificus* using the direct viable count.

	DVC
O VBNC	0.165
B VBNC	0.173
T VBNC	0.147

In addition to the increased resistance of the VBNC cells of *V. vulnificus*, the resuscitation patterns after irradiation were examined. It was determined that complete resuscitation occurred after 48 hours of room temperature incubation, with levels matching the original direct viable count, but not higher. Resuscitation was guaranteed in this case by the addition nalidixic acid to the resuscitation medium, which prevented the growth of any culturable cells that may have been present in the sample. The plate counts after 48 hours at room temperature were again equal to the DVC, but not higher.

The type of resuscitation media and microbial concentration during resuscitation were also examined. After the irradiation of VBNC cells (O, T and B), 2.0 mL undiluted samples were allowed to resuscitate at room temperature for 48 hours, as well as 10-, 100- and 1000-fold dilutions. The undiluted samples resuscitated to the original DVC values, but none of the diluted samples resuscitated. Full microcosms of VBNC cells were harvested by centrifugation, washed 2X, and resuspended for resuscitation in fresh ASW. It was determined that complete resuscitation occurred in the fresh seawater. However, when diluting the fresh ASW 10-, 100- and 1000-fold, no resuscitation occurred. This indicates that the resuscitation media (old ASW) is not harboring vital nutrients that induce resuscitation, because new ASW allows for resuscitation. The focus is probably properly placed on the actual cell concentration, as there appears to be a cooperative effect among the cells as concentrations below 10^5 per mL would not resuscitate.

In conclusion, irradiation processing cannot be considered as a method to sterilize shellstock oysters, and provide a shelf stable product. Irradiation can reduce some pathogens and viruses, perhaps below their infective dose, but not rid the shellstock oyster completely of all contaminants. The shellstock oyster poses many challenges to irradiation and food processing technology. These problems include uneven dose distribution, different shell to meat ratios for oysters from different geographic locations and the potential for growback of organisms in the irradiated product over time. Furthermore, oysters are lives animals with their own inherent radiation sensitivity, and thus radiation D value. It is clear that the D_{10} value of oysters falls somewhere in the range where bacterial and viral reduction is observed. Thus, the conundrum of deciding on the dose that will give the best shelf life and maximum bacterial reduction will continue. The survival of organisms is a great concern because when competition is altered between the flora, the result could be the rapid outgrowth of a potentially dangerous microbe.

Concerning the irradiation of V. vulnificus, it is apparent that in simple media like ASW, it is a very radiosensitive microorganism. However, in a complex system like a shellstock oysters, there is a protective effect by the shell itself and this organism can survive. More importantly it can grow and divide in dry cold storage, or even worse enter the viable but nonculturable state. The VBNC forms of V. vulnificus are 3X more resistant to radiation than the corresponding culturable forms and this too could be a potential problem in winter harvest oysters that may have VBNC cells. There is evidence from this research for the presence of resuscitation of VBNC V. vulnificus cells post-irradiation and that cell concentration plays a key role in the resuscitation process.

APPENDIX

MICROBIOLOGICAL CONSEQUENCES

Table A. The Microbiological Analysis of FL (A0, A1, A3) and TX (T0, T1 and T3) Shellstock Oysters Irradiated at 0, 1.0 and 3.0 kGy and then Kept Under Dry Storage at 4-6°C Over a Two Week Time Period.

Day 0	PCA	PCAS	MPN	TCBS	CPC	LB	EC-MUG
A0	10,900	16,500	23,000	23,000	23,000	210	210
A1	2050	950	4900	4900	4900	330	330
A3	30	85	2300	2300	2300	63	0
T0	18,500	18,000	23,000	23,000	23,000	230	230
T1	15	65	4900	4900	4900	200	200
T3	30	30	2300	2300	2300	0	0

Day 2	PCA	PCAS	MPN	TCBS	CPC	LB	EC-MUG
A0	11,900	11,400	49,000	49,000	49,000	490	490
A1	8050	9,400	7900	7900	7900	130	130
A3	1550	305	4900	4900	4900	45	0
T0	15,600	29,000	49,000	49,000	49,000	490	490
T1	2500	3950	23,000	23,000	23,000	490	490
T3	380	1330	7900	7900	7900	490	490

Day 4	PCA	PCAS	MPN	TCBS	CPC	LB	EC-MUG
A0	68,000	185,000	230,000	49,000	33,000	2300	2300
A1	7050	11,400	23,000	13,000	13,000	1300	1300
A3	6550	5100	23,000	13,000	13,000	1300	130
T0	23,500	39,000	230,000	49,000	49,000	1300	1300
T1	3300	5250	23,000	13,000	13,000	790	790
T3	505	1830	23,000	2000	2000	230	130

Day 7	PCA	PCAS	MPN	TCBS	CPC	LB	EC-MUG
A0	50,500	230,000	490,000	49,000	49,000	2300	2300
A1	5000	3300	33,000	13,000	13,000	230	130
A3	5500	5650	23,000	13,000	13,000	490	490
T0	181,000	201,000	490,000	230,000	130,000	2300	2300
T1	165,000	157,000	230,000	230,000	23,000	330	230
T3	31,000	49,000	23,000	23,000	23,000	230	230

PCA= plate count agar, PCAS = plate count agar + 2.5% NaCl, MPN = alkaline peptone water most probable number, TCBS = thiosulfate citrate bile salts sucrose agar, CPC= colistin polymixin cellobiose agar, LB = Lactose Broth and EC-MUG = *E. coli* broth with MUG

Table A.--continued.

Day 9	PCA	PCAS	MPN	TCBS	CPC	LB	EC-MUG
A0	60,000	237,000	490,000	49,000	49,000	230	230
A1	22,500	174,000	49,000	49,000	33,000	330	130
A3	25,000	239,000	[illegible],000	49,000	33,000	230	230
T0	220,000	255,000	230,000	230,000	230,000	230	230
T1	95,000	345,000	130,000	130,000	73,000	230	230
T3	14,500	16,100	23,000	23,000	23,000	230	230
Day 11	**PCA**	PCAS	MPN	TCBS	CPC	LB	EC-MUG
A0	54,000	207,000	490,000	73,000	73,000	330	330
A1	14,500	177,000	49,000	49,000	49,000	230	230
A3	21,000	177,000	23,000	23,000	23,000	230	130
T0	265,000	190,000	110,000	110,000	110,000	330	330
T1	134,000	220,000	70,000	70,000	70,000	230	130
T3	17,000	21,500	23,000	23,000	23,000	230	130
Day 14	**PCA**	PCAS	MPN	TCBS	CPC	LB	EC-MUG
A0	63,000	190,000	490,000	230,000	230,000	700	700
A1	24,000	177,000	230,000	230,000	230,000	330	330
A3	15,000	136,000	49,000	49,000	23,000	230	230
T0	234,000	188,000	230,000	230,000	230,000	1400	1400
T1	165,000	162,000	170,000	170,000	170,000	330	130
T3	20,000	29,000	23,000	23,000	23,000	230	130

PCA= plate count agar, PCAS = plate count agar + 2.5% NaCl, MPN = alkaline peptone water most probable number, TCBS = thiosulfate citrate bile salts sucrose agar, CPC= colistin polymixin cellobiose agar, LB = Lactose Broth and EC-MUG = E. coli broth with MUG

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BIOGRAPHICAL SKETCH

Dustin William Dixon, the younger of Albert and Joyce Dixon's two children, was born July 6, 1968, in Tampa, Florida. He attended St. John's Parish Day School, and graduated with honors from Tampa Preparatory High School in 1986. He was awarded his Bachelor of Science in Agriculture (BSA) degree from the University of Florida in May, 1990, from the Department of Microbiology and Cell Science. He changed departments at the University of Florida for graduate study, to the Department of Food Science and Human Nutrition, in pursuit of his Master of Science degree under the supervision of Dr. Gary E. Rodrick. He was awarded the Master of Science degree in August of 1992. Furthermore, he was initiated into the honorary society of agriculture, Gamma Sigma Delta. He was awarded a .33 FTE assistantship to continue graduate study towards a Doctor of Philosophy degree in the Department of Food Science and Human Nutrition. He was awarded a Ph.D. in August of 1996, and plans to begin a career in either the food industry or academia.